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# Studying the Effects of Ultrasound Shock on Cell Wall Permeability and Survival of Some LAB in Milk

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**Abstract:** Microstructure changes and micro-damage behavior of some LAB under the impact of ultrasound shock (20 kHz for 5, 10, 15, 20, 25 and 30 minutes) was studied by optical microscope and Transmission Electron Microscope (TEM). Three kinds of micro damages are usually produced by ultrasound, micro-cracks, micro-voids and ruptures.Studies, by TEM showed that ultrasound can increase the cell wall permeability of the cells, which is important in the release of enzymes such as ß-galactosidas reduction of the coagulation time. The survival of LAB was very low in very long exposures of ultrasound.

**Key words:** Cell wall permeability . LAB . ultrasound shock . survival

## INTRODUCTION

Probiotic bacteria are defined as living micro-organisms, which upon ingestion in certain numbers; exert health benefits beyond inherent basic nutrition. Probiotics ability to grow well in the product and also their survival in the final product is of great importance to show their health benefits. Increasing the enzymatic activity of probiotics without any negative effect on their survival is noteworthy [1]. The secretive enzymes of probiotic bacteria improve their survival [2]. Every kind of shock can influence the functionality of the cell wall of bacteria and improve or damage its normal physiological and vital activities [3, 4].

Ultrasound causes cavitation in aqueous solutions, which is an effective factor in damaging the cell wall of the micro-organisms [5]. When a bubble collapses, a strong shear rate is generated in the environment that breaks the chemical bounds in the cells' wall and membranes' [6]. Depending on the strength and frequency of waves, cell wall structure and sonication environment, the impact of ultrasound would be different.

## MATERIAL AND METHODS

Milk with 2/5% fat and 10/5% dry weight was supplied from local market. Pure lyophilized cultures of Lactobacillus acidophilus (strain, LAI), Lactobacillus casei (strains AB) Lactococcus lactis.spp cremoris and lactococus lactis.spp lactis were supplied from local industries. Then the packages of bacteria were prepared according to the company's instructions and were added (5%, v/w) to the milk aseptically, then the milk was distributed into tubes with screwing caps to perform the shocks. Ultrasound shock for 5, 10, 15, 20, 25, 30 minutes in the constant frequency of 20 KHz, amplitude of 80% and time cycle of 0.5 minutes were performed on the samples, then, the samples were prepared to observe under the optical and Transmission Electron Microscope.

## RESULTS AND DISCUSSIONS

Figure 1- 4 show the effect of ultrasound shock on probiotic bacteria studied by optical microscope. Results showed that in exposure duration less than 20 minutes, colonies of bacteria grew on the media, but their sizes were very small. Increasing the exposure

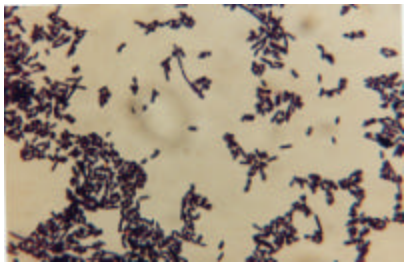

Fig. 1: Microscopic picture of probiotic bacteria before ultrasound shock. The number of Lactococcus and lactobacillus are the same and all of the bacteria are safe

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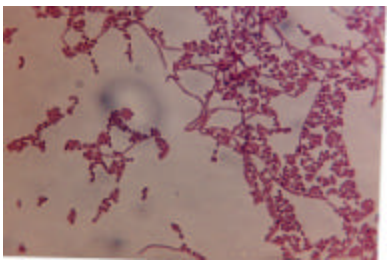

Fig. 2: Microscopic picture of probiotic bacteria after 10 mintes. The reduction of population is obvious

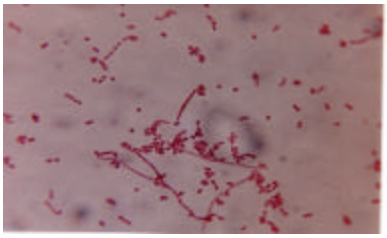

Fig. 3: Microscopic picture of probiotic bacteria after 20 minutes

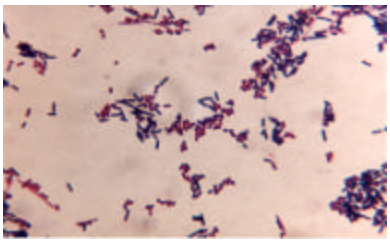

Fig. 4: Microscopic picture of probiotic bacteria After 30 minutes (filaments are compeletly formed)

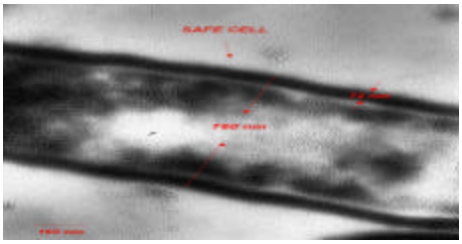

Fig. 5: Microscopic picture of Lactobacillus before ultra sound shock (the cell wall is safe and the diameter is 760 nm)

duration caused the lactobacillus to adhere together and develope streptobacillus or filaments, this could be due the activation of some special proteins (Frz) in the cell wall of the bacteria. Results showed that in comparison to lactococcus, lactobacillus were more affected by ultrasound shock, the reduction of lactobacillus population was obvious in different stages. Figure 8-15 show the effect of ultrasound shock on probiotic bacteria studied by TEM. Depending on the duration of exposure, Micro-cracks, Micro-voids and raptures were formed on the cell wall. In short exposure times, bacteria recover damages like micro-cracks and micro-voids, but in longer exposure times, rupture happens. Damages, induce the release of intracellular compounds, enzymes, polysaccharides and polymers to the environment.

Table 1 shows the changes of pH, acidity and total count of bacteria after the exposure to the ultrasound.

## CONCLUSIONS

1-Cell wall permeability, volume and size of the cells are affected by ultrasound in different ways (Fig. 6, 8, 10).

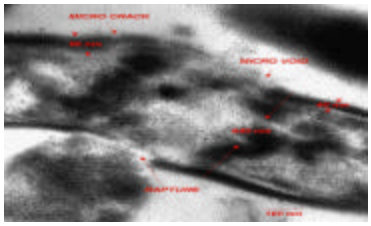

Fig. 6: Microscopic picture of Lactobacillus after 20 minutes ultra sound shock. Rupture, micro-cracks and micro-voids are observed and the diameter reduced

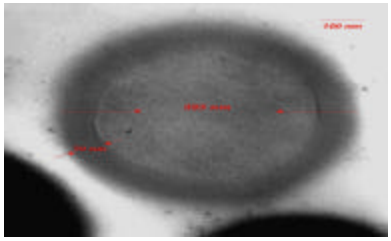

Fig. 7: Microscopic picture of Lactobacillus lactococcus lactis spp lactis before ultrasound shock (the cell wall is safe and the diameter is 525 nm)

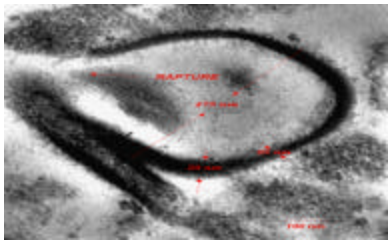

Fig. 8: Microscopic picture of Lactobacillus after 30 minutes (cell wall is raptured and diameter is reduced to 475 nm)

Table 1: Changes of pH, acidity and total count of bacteria after the exposure to the ultrasound.

| | 0 min | 5 min | 10 min | 15 min | 20 min | 25 min | 30 min |
|---|---|---|---|---|---|---|---|
| pH | 6.4 | 5.1 | 4.99 | 4.98 | 4.8 | 4.2 | 4.2 |
| Acidity | 0.2 | 0.28 | 0.29 | 0.31 | 0.32 | 0.3 | 0.3 |
| Total count | 5.98 CFU | 5.80 CFU | 5.70 CFU | 5.52 CFU | 5.24 CFU | 5.14 CFU | 4.99 CFU |
| Dry matter | 12.30 | 12.10 | 12.30 | 12.00 | 12.10 | 12.10 | 12.10 |

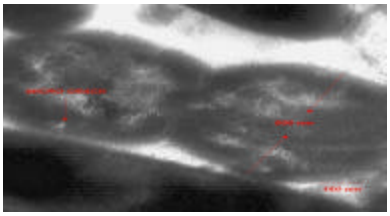

Fig. 9: Microscopic picture of Lacctococcus lactis spp cremoris (the cell wall is safe with specified diameter)

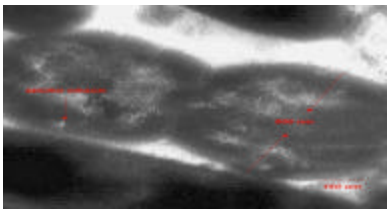

Fig. 10: Microscopic picture of Lactobacillus lactis spp cremoris after 20 minutes (the diameter is reduced and micro-crack or micro-void could be observed)

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